Microparticle-Based Cellular Arrays For Vaccine Development And Optimization

Abhinav. Acharya[1], Natalia. Dolgova[2] and Benjamin.G. Keselowsky[2]
[1]University of Florida, Material Science and Engineering, Gainesville, FL, USA ;
[2]University of Florida, Pruitt Family Department of Biomedical Engineering, Gainesville, FL, USA

Introduction

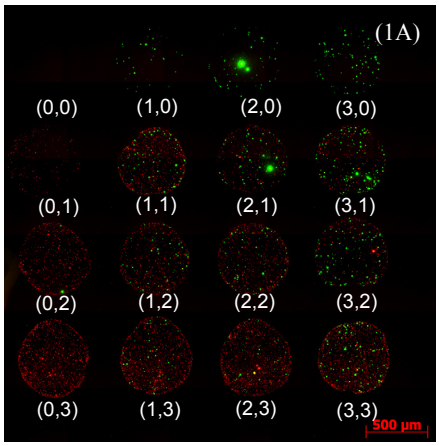
The use of non-living vaccines (e.g., synthetic peptides derived from antigenic epitopes) provides multiple advantages over live vaccines. Properly designed biomaterial vehicles for vaccines can help in improving the efficacy of targeting and therapeutic effect. However, although there are now scores of known antigenic epitopes and adjuvants, there has not emerged a method with which to examine their functional effects on immune cells in a high-throughput manner. We are utilizing high-throughput cellular microarrays in order to optimize microparticle-based vaccine formulations amenable to needle-free immunization (e.g., oral or epidermal powder immunization). Specifically, we aim to optimize combinations of antigenic peptides, antigen presenting cell-targeting molecules, immuno-functional molecules (e.g., "danger signals" targeting toll-like receptors or tolerogenicity-inducers) as well as chemoattractants to improve antigen-presenting cell recruitment into the vaccination site.

Materials and Method

Immature DCs (iDCs) were obtained by culturing precursors isolated from bone marrow of C57BL/6j mouse in the presence of 20 ng/ml of growth factor GM-CSF for 10 days with half-media change every alternate day. Microparticles with differential concentrations of immunotherapeutic molecules or fluorescent dyes loaded 50:50 PLGA, 1-5 micron microparticles (MPs) were manufactured using water in oil in water type emulsion. Encapsulation efficiency was determined. These MPs were then printed on cell-adhesive islands with non-fouling background. Immature DCs were selectively seeded onto islands. Pro- and anti- inflammatory cytokines and surface markers indicating activation of DCs were quantified using immunofluoroscent staining. Lenti-virus (mbanana and mtangerine) were printed to emphasize the modularity of the technique.

Results

Bio-degradable MPs loaded with bioactive factors providing timed-release can be arrayed in a combinatorial fashion to optimize dosing and investigate response to multiple factors. Microparticle loading and delivery is quantitative and tightly controlled (**Figure 1A, B, C**). Arrayed spots of dendritic cells are co-localized with MPs loaded with bioactive factors. Cells are selectively adherent onto spots (**Figure 2A, B**), while off-spot surface is non-fouling. The number of DCs and MPs on each spot were quantified using image analysis and DC to MP ration can be controlled (**Figure 2C**). Dendritic cell activation was probed through immuno-staining for markers of activation. Shown is an array of DCs and MPs which have been loaded with activating factor, LPS, and immuno-stained for markers of activation, MHC-II and CD86 (**Figure 3**). Cytokine production of IL-10 and IL-12 was similarly quantified in situ through a "golgi-stop" treatment, followed by immuno-staining for intracellular cytokines (**Figure 4A, B**). Relative levels of cellular response are quantified in situ through either image analysis, or laser microarray scanner. Scale bar is 500 µm. Lentivirus transfected DCs expressing mbanana protein was quantified using image analysis (**Figure 5A, B**).



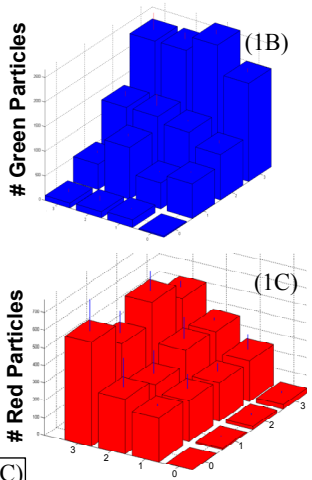


Figure 1: **A**) Orthogonal array of FITC and rhodamine loaded MPs. **B&C**) Image analysis quantification of MPs.

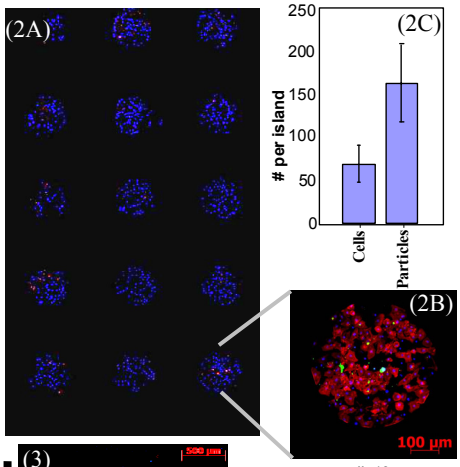


Figure 2: **A**) DCs selectively adhere on islands at 10X (blue-nucleus; red-MPs). **B**) Single island of co-localized DCs and MPs at 40X (red-actin, green-MPs, blue-nucleus). **C**) Quantification of number of MPs and DCs.

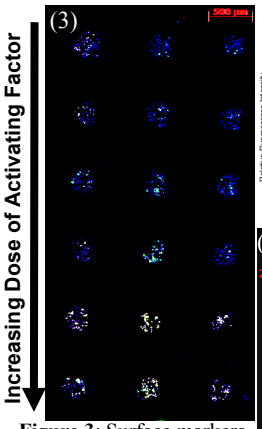


Figure 3: Surface markers on DCs. blue-nucleus, red-MHC-II, green-CD86.

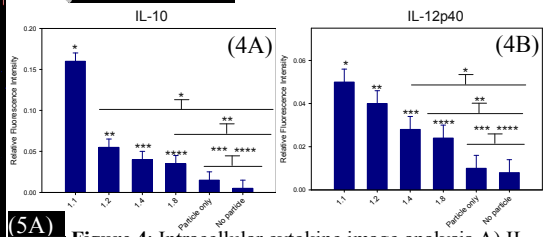


Figure 4: Intracellular cytokine image analysis A) IL-10 B)IL-12.

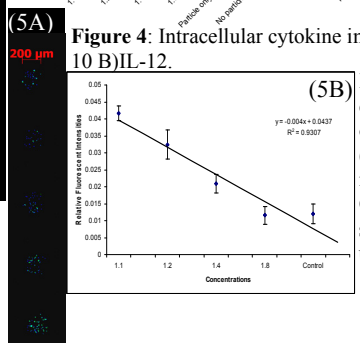


Figure 5: **A**) Single column of an array of transfected DCs (blue-nucleus, green-mbanana protein). **B**) Quantification of surface expression using image analysis.

Conclusions

We successfully constructed a technique to characterize DC-activation to vaccine formulations in a highthroughput manner. We were able to quantify and modulate activation and maturation of DCs in situ.

Reference: Janeway et al *Immunobiology* (2005); Waeckerle-Men Y, Groettrup M *Adv Drug Deliv Rev* (2005); Elamanchili P et al *Vaccine* (2004); Yoshida M *J Biomed Mater Res A* (2007).